SUPPLEMENTAL INFORMATION

Number of Supplemental Tables: 2

Number of Supplemental Figures: 8

Table S1. Primer sequences for qPCR
Related to STAR METHODS "RNA extraction and quantitative PCR (qPCR)"

Gene	Forward (5`- 3`)	Reverse (5`- 3`)
Aldh1l1	GCAGGTACTTCTGGGTTGCT	GGAAGGCACCCAAGGTCAAA
Amigo2	GAGGCGACCATAATGTCGTT	GCATCCAACAGTCCGATTCT
Apoe	ACAGATCAGCTCGAGTGGCAAA	ATCTTGCGCAGGTGTGTGGAGA
Aqp4	CTTTCTGGAAGGCAGTCTCAG	CCACACCGAGCAAAACAAAGAT
Aspg	GCTGCTGGCCATTTACACTG	GTGGGCCTGTGCATACTCTT
Axl	AGGCTCATTGGCGTCTGTT	ATCGCTCTTGCTGGTGTAG
B3gnt5	CGTGGGGCAATGAGAACTAT	CCCAGCTGAACTGAAGAAGG
C1qa	AAAGGCAATCCAGGCAATATCA	TGGTTCTGGTATGGACTCTCC
C4b	ACTTCAGCAGCTTAGTCAGGG	GTCCTTTGTTTCAGGGGACAG
C3	AAG CAT CAA CAC ACC CAA CA	CTT GAG CTC CAT TCG TGA CA
C3aR	TCGATGCTGACACCAATTCAA	TCCCAATAGACAAGTGAGACCAA
Ccl2	GAAGGAATGGGTCCAGACAT	ACGGGTCAACTTCACATTCA
Ccl5	GTGCCCACGTCAAGGAGTAT	CCACTTCTTCTCTGGGTTGG
CD14	GGACTGATCTCAGCCCTCTG	GCTTCAGCCCAGTGAAAGAC
CD68	ACTGGTGTAGCCTAGCTGGT	CCTTGGGCTATAAGCGGTCC
Clec7a	CATCGTCTCACCGTATTA ATGCAT	CCCAGAACCATGGCCCTT
Clu	GTAGGAGTGTCTGGGAGGGA	GCAAGTGCAGGCATTAGTGT
Csf1	AAGGGACTCACTAGCCTGGA	ATCAGGCTCTCTTCTTGGGA
Cx3cr1	CAGCATCGACCGGTACCTT	GCTGCACTGTCCGGTTGTT
Cxcl10	CCCACGTGTTGAGATCATTG	CACTGGGTAAAGGGGAGTGA
Fbln5	CTTCAGATGCAAGCAACAA	AGGCAGTGTCAGAGGCCTTA
Fli1	GACCAACGGGGAGTTCAAAATGACG	GGAGGATGGGTGAGACGGGACAAAG
Gbp2	GGGGTCACTGTCTGACCACT	GGGAAACCTGGGATGAGATT
Gfap	AGAAAGGTTGAATCGCTGGA	CGGCGATAGTCGTTA
Iba1	CAGACTGCCAGCCTAAGACA	AGGAATTGCTTGTTGATCCC
IL1β	GCAACTGTTCCTGAACTCAACT	ATCTTTTGGGGTCCGTCAACT
IL6	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC
Irf8	CGTGGAAGACGAGGTTACGCTG	GCTGAATGGTGTGTGTCATAG
Itgax	CTGGATAGCCTTTCTTCTGCTG	GCACACTGTGTCCGAACTCA
Itgam	CCATGACCTTCCAAGAGAATGC	ACCGGCTTGTGCTGTAGTC
Nfe2l2	GCCTTACTCTCCCAGTGAATAC	CCCAAATGGTGCCTAAGA

Nfkb1	GAAATTCCTGATCCAGACAAAAAC	ATCACTTCAATGGCCTCTGTGTAG
Osmr	GTGAAGGACCCAAAGCATGT	GCCTAATACCTGGTGCGTGT
P2ry12	AAAATGCCTGCTGCTTGAAT	TGAAGAAATTCCAACAAAACGA
Pax6	TTTAACCAAGGGCGGTGAGCAG	TCTCG-GATTTCCCAAGCAAAGATG
Ptx3	AACAAGCTCTGTTGCCCATT	TCCCAAATGGAACATTGGAT
Runx1	CCTGGAGGATGTCCTTTCAA	CTGGATCTGCCTGGCAT
S100β	CTCTCACTTCCTGGAGGAAATC	AAGAACTCATGACAGGCTGTGG
Serping1	ACAGCCCCCTCTGAATTCTT	GGATGCTCTCCAAGTTGCTC
Serpina3n	CCTGGAGGATGTCCTTTCAA	TTATCAGGAAAGGCCGATTG
SOCS3	GCTCCAAAAGCGAGTACCAGC	AGTAGAATCCGCTCTCCTGCAG
Spi1	AATTTGCCTGCCCTGAGTGC	TTGGACCCATGCTACCTTGC
Stat1	CTGAATATTTCCCTCCTGGG	TCCCGTACAGATGTCCATGAT
Stat3	CAGAAAGTGTCCTACAAGGGCG	CGTTGTTAGACTCCTCCATGTTC
Stat5a	CGCTGGACTCCATGCTTCTC	GACGTGGGCTCCTTACACTGA
Stat5b	GGACTCCGTCCTTGATACCG	TCCATCGTGTCTTCCAGATCG
Tmem119	GTGTCTAACAGGCCCCAGAA	AGCCACGTGGTATCAAGGAG
Tmsf4	GCCCAAGCATATTGTGGAGT	AGGGTAGGATGTGGCACAAG
TNFα	CCCTCACACTCAGATCATCTTCT	GCTACGACGTGGGCTACAG
Trem2	GACCTCTCCACCAGTTTCTCC	TACATGACACCCTCAAGGACTG
Tyrobp	TGGTGTTGACTCTGCTGATTGC	CCTTCCGCTGTCCCTTGAC
hGAPDH	AGGGCTGCTTTTAACTCTGGT	CCCCACTTGATTTTGGAGGGA
hC3	TCACCGTCAACCACAAAGCTGCTACC	TTTCATAGTAGGCTCGGATCTTCCA
hC3aR	GCCCACTGGATAACTCTGATGC	TGATCGTCATCTGTGAATTGGC
hSpi1	GAGAAGCTGATGGCTTGGAG	GGCGAATCTTTTTCTTGCTG
hStat1	CTAGTGGAGTGGAAGCGGAG	CACCACAAACGAGCTCTGAA
hStat3	GGCATTCGGGAAGTATTGTCG	GGTAGGCGCCTCAGTCGTATC
hStat5a	CTCCAGTGCAGCTCTCCG	CCTCAGGCTCTCCTGGTACT
hStat5b	CCCTGTGAGCCCGCAACTGCG	TGACTGTGCGTGAGGGATCCA
hNfkb1	CACTGCTCAGGTCCACTGTC	CTGTCACTATCCCGGAGTTCA
hRunx1	CTGCTCCGTGCTGCCTAC	AGCCATCACAGTGACCAGAGT
hFli1	CCACTAGTTACCCACCCCAAACTG	GTGATACAGCTGGCGTTGGCG
hPax6	TCTTTGCTTGGGAAATCCG	CTGCCCGTTCAACATCCTTAG
hNfe2l2	TGGTTGCCTCTCACTACCCATTGT	GCTTGTGCTGCCATCGAGTGATTT
hIrf8	CCAGATTTTGAGGAAGTGACG	TGGGAGAATGCTGAATGGTGC
hMAPT	GTCGAAGATTGGGTCCCT	GACACCACTGGCGACTTGTA

Table S2. Human demographics
Related to STAR METHODS "Human subjects"

Diagnosis	Sex	Age
NCI (used for staining)	F	68
NCI	F	65
NCI	F	67
NCI	M	59
NCI	F	59
NCI	F	65
AD (used for staining)	M	64
CBD (used for staining)	F	66
CBD	M	66
CBD	M	52
CBD	M	61
CBD	M	44
Pick`s	M	57
Pick`s	M	71
Pick`s	M	72
Pick`s	M	59
Pick`s	M	62
PSP	M	70
PSP	M	84
PSP	F	71
PSP	M	71
PSP	F	77

NCI – non-cognitive impairment; AD – Alzheimer`s disease; CBD –corticobasal degeneration; Pick`s – Pick`s disease; PSP – progressive supranuclear palsy.

Supplemental Figures and Figure Legends

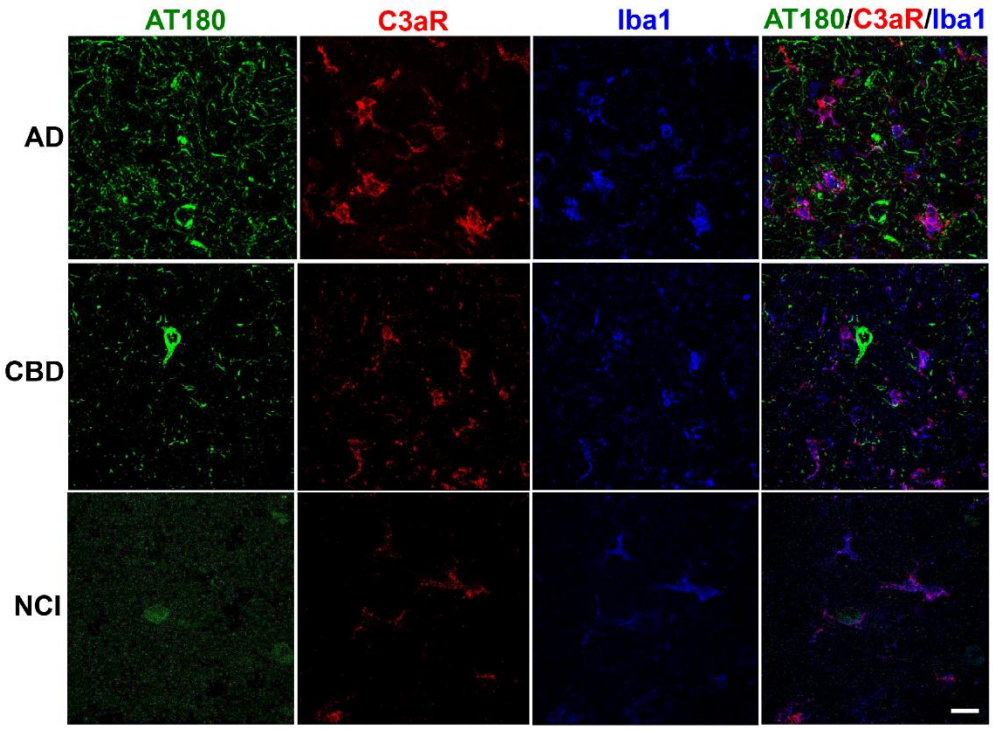


Figure S1. Related to Figure 1. Examples of co-immunostaining of MFC of AD, CBD, and NCI brains using anti-phospho-tau (AT180), -C3aR and -Iba1 antibodies. Scale bar: 100 µM.

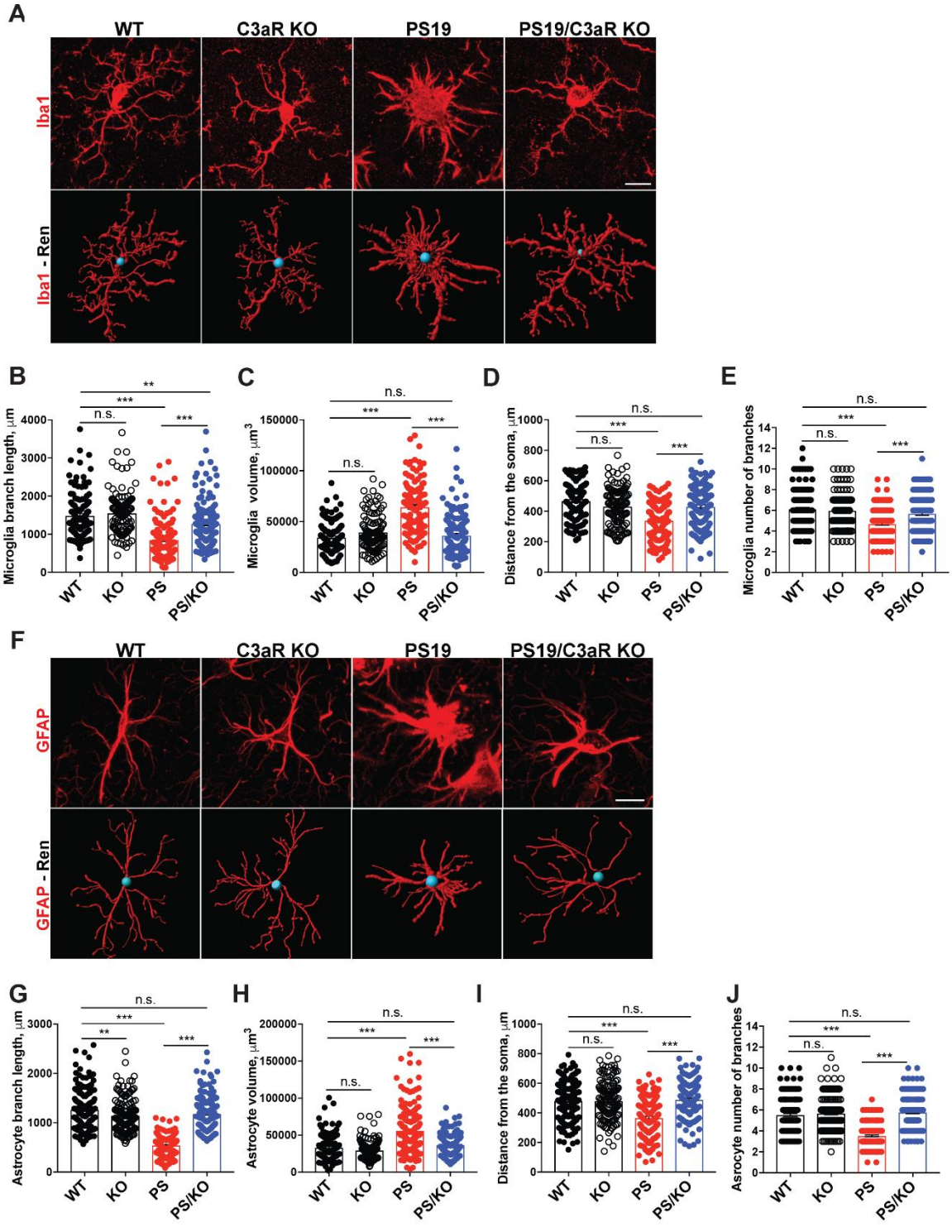

A
WT
C3aR KO
PS19
PS19/C3aR KO
Iba1
Iba1 - Ren
B
Microglia branch length, μm
C
Microglia volume, μm3
D
Distance from the soma, μm
E
Microglia number of branches
F
GFAP
GFAP - Ren
G
Astrocyte branch length, μm
H
Astrocyte volume, μm3
I
Distance from the soma, μm
J
Asrocyte number of branches
n.s.
WT
KO
PS
PS/KO

Figure S2. Related to Figure 2. Quantification of microglia and astrocyte morphologies in PS19 mice.

(A) Representative Iba1 immunostaining (upper panel) and 3D rendering (lower panel, Iba1-Ren) of WT, C3aR KO, PS19, and PS19/C3aR KO brain sections at 9 months. Scale bar: 5 µM.

(B - E) Quantification of microglia branch length (B), cell volume (C), branch distance from the soma (D), and branch number (E). n=6/genotype, N=20-30 cells/animal. One-way ANOVA followed by Sidak`s HSD test. n.s.: non-significant; ** $p<0.01$; *** $p<0.001$. Data is presented as mean ± s.e.m.

(F) Representative GFAP immunostaining (upper panel) and 3D rendering (lower panel, GFAP-Ren) of WT, C3aR KO, PS19, and PS19/C3aR KO mice at 9 months. Scale bar: 5 µM.

(G - J) Quantification of astrocyte branch length (G), cell volume (H), branch distance from the soma (I), and branch number (J). n=6/genotype, N=20-30 cells/animal. One-way ANOVA followed by Sidak`s HSD test. n.s.: non-significant; ** $p<0.01$; *** $p<0.001$. Data is presented as mean ± s.e.m.

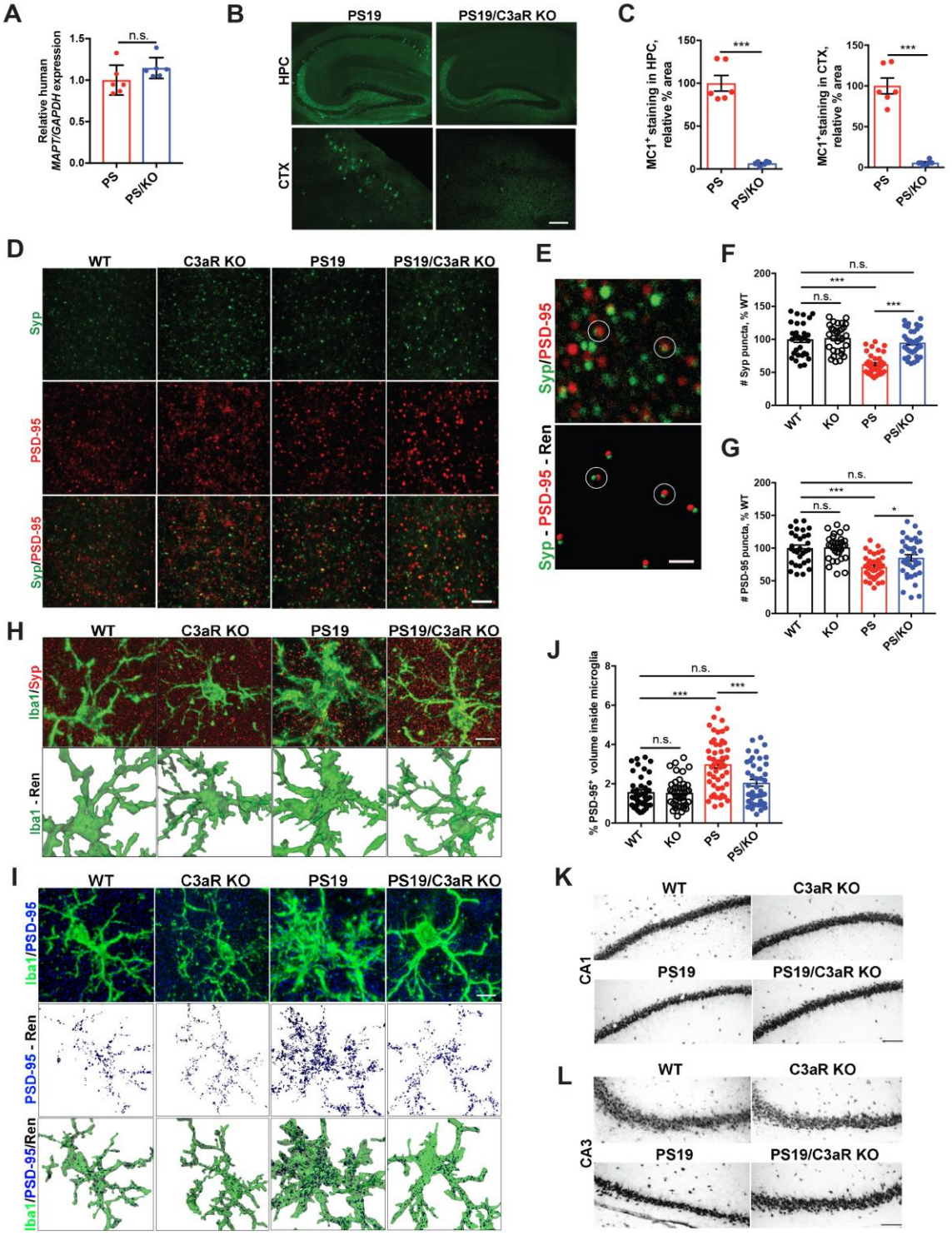

A
Relative human MAPT/GAPDH expression
n.s.
PS
PS/KO
B
PS19
PS19/C3aR KO
HPC
CTX
C
MC1+ staining in HPC, relative % area
MC1+ staining in CTX, relative % area
***
PS
PS/KO
D
WT
C3aR KO
PS19
PS19/C3aR KO
Syp
PSD-95
Syp/PSD-95
E
Syp/PSD-95
Syp - PSD-95 - Ren
F
Syp puncta, % WT
n.s.
***
WT
KO
PS
PS/KO
G
PSD-95 puncta, % WT
*
H
Iba1/Syp
Iba1 - Ren
J
% PSD-95+ volume inside microglia
I
Iba1/PSD-95
PSD-95 - Ren
Iba1/PSD-95/Ren
K
CA1
L
CA3

Figure S3. Related to Figure 3 and Figure 4. Effect of *C3ar1* deletion on tau pathology and synaptic and neuronal properties in PS19 mice.

(A) qPCR analysis of human *MAPT* mRNA levels in 9 month-old PS19 and PS19/C3aR KO mice. n=6/genotype, Student's t-test. n.s.: non-significant.

(B, C) Representative MC1 immunostaining (B) and its quantification (C) in hippocampus (HPC) and cortex (CTX) of 9 month-old PS19 and PS19/C3aR KO mice. Scale bar: 0.5 mm. n=6/genotype. Student's t-test. n.s.: non-significant; ***p<0.001. Data is presented as mean ± s.e.m.

(D) Representative Syp, PSD-95 or merged images in CA3 area of hippocampus of WT, C3aR KO, PS19, and PS19/C3aR KO mice at 9 months. Scale bar: 5 µM.

(E) Representative high magnification confocal images of Syp and PSD-95 co-immunostaining in CA3 area of hippocampus of PS19 mice (upper) and 3D reconstruction (lower) of co-localized puncta (circled) using Imaris software. Scale bar: 2.5 µM.

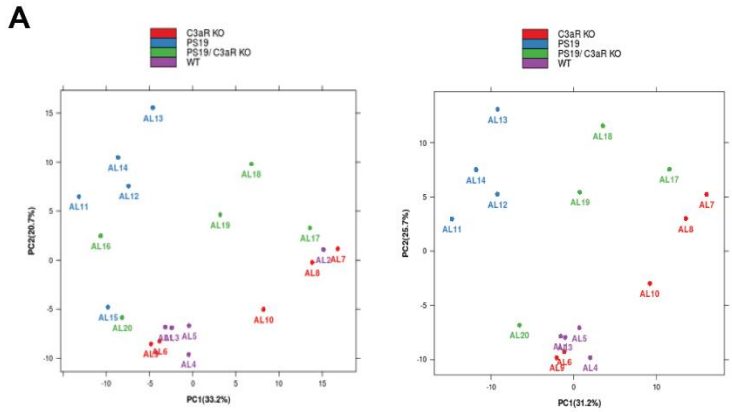
(F, G) Quantification of the number of Syp-positive presynaptic puncta (F) or PSD-95-positive postsynaptic puncta (G) in CA3 area of hippocampus of WT, C3aR KO, PS19, and PS19/C3aR KO mice at 9 months (C) (mean ± s.e.m). n=6/genotype with 5-7 planes per mouse. One-way ANOVA followed by Sidak`s HSD test. n.s.: non-significant; *p<0.05; ***p<0.001.

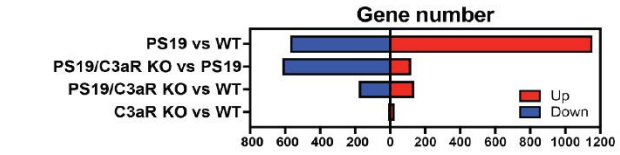
(H) Representative Iba1 and Syp co-immunostaining (Iba1/Syn) and Imaris 3D rendering of Iba1-positive microglia (Iba1-Ren) of WT, C3aR KO, PS19, and PS19/C3aR KO mice. Scale bar: 5 µM.

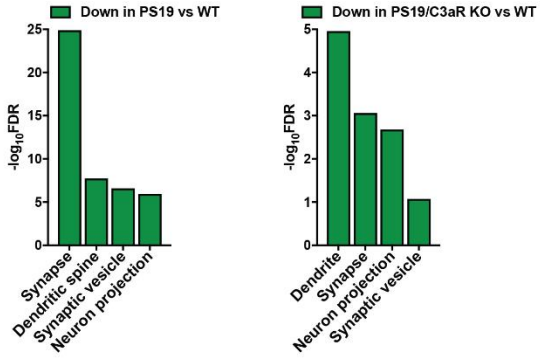
(I) Representative Iba1 and PSD-95 co-immunostaining (Iba1/PSD-95) and 3D reconstruction and rendering of PSD-95 signals inside Iba1-positive microglia (Iba1/PSD-95/Ren) from 9 month-old mice using the Imaris software. Scale bar: 5 µM.

(J) Quantification of (I) (mean ± s.e.m). n=6/genotype; 10-15 cells/mouse were quantified. One-way ANOVA followed by Sidak`s HSD test. n.s.: non-significant; ***p<0.001.

(K, L) Enlarged NeuN staining images of CA1 (E) and CA3 (F) areas of hippocampus of 9 month-old WT, C3aR KO, PS19 and PS19/C3aR KO mice selected for unbiased stereology. Scale bar: 50 µM.


A
C3aR KO
PS19
PS19/ C3aR KO
WT
PC1(33.2%)
PC2(20.7%)
PC1(31.2%)
PC2(25.7%)


B
Gene number
PS19 vs WT
PS19/C3aR KO vs PS19
PS19/C3aR KO vs WT
C3aR KO vs WT
Up
Down


C
Down in PS19 vs WT
Down in PS19/C3aR KO vs WT
-log10FDR
Synapse
Dendritic spine
Synaptic vesicle
Neuron projection
Dendrite

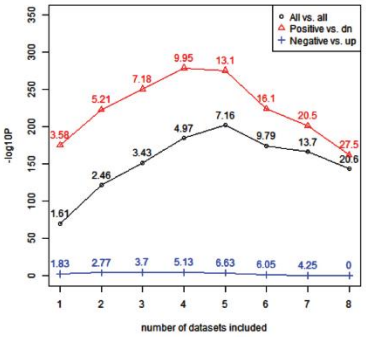

D
All vs. all
Positive vs. dn
Negative vs. up
-log10P
number of datasets included

Figure S4. Related to Figure 5. Analysis of mouse RNAseq results and human AD datasets.

(A) PCA analysis showing clustering of the 20 animals (AL1-20) in 3 different groups based on gene expression (left plot). Mice AL2, AL15 and AL16 were removed from future analysis based on PCA clustering (right plot).

(B) The number of DEGs by pairwise genotype comparisons: PS19 vs WT; PS19/C3aR KO vs PS19; PS19/C3aR KO vs WT and C3aR KO vs WT. FDR<0.01.

(C) GO term analysis of the 571 DEGs significantly downregulated in PS19 vs WT and PS19/C3aR KO vs WT. FDR<0.01.

(D) The enrichment of PS19/C3aR KO DEG signatures in the consensus *C3AR1*-correlated gene signatures. X axis indicates the number of datasets in which a particular gene expression significantly correlated with *C3AR1*. The number on the top of each dot represents fold enrichment. Black line represents the overall enrichment regardless of correlation direction. Red line represents the intersection between the gene signatures that are positively correlated with *C3AR1* in human brain and the downregulated genes in PS19/C3aR KO mice. Blue line indicates the enrichment between the genes negatively correlated with *C3AR1* in human brain and the upregulated genes in PS19/C3aR KO mice.

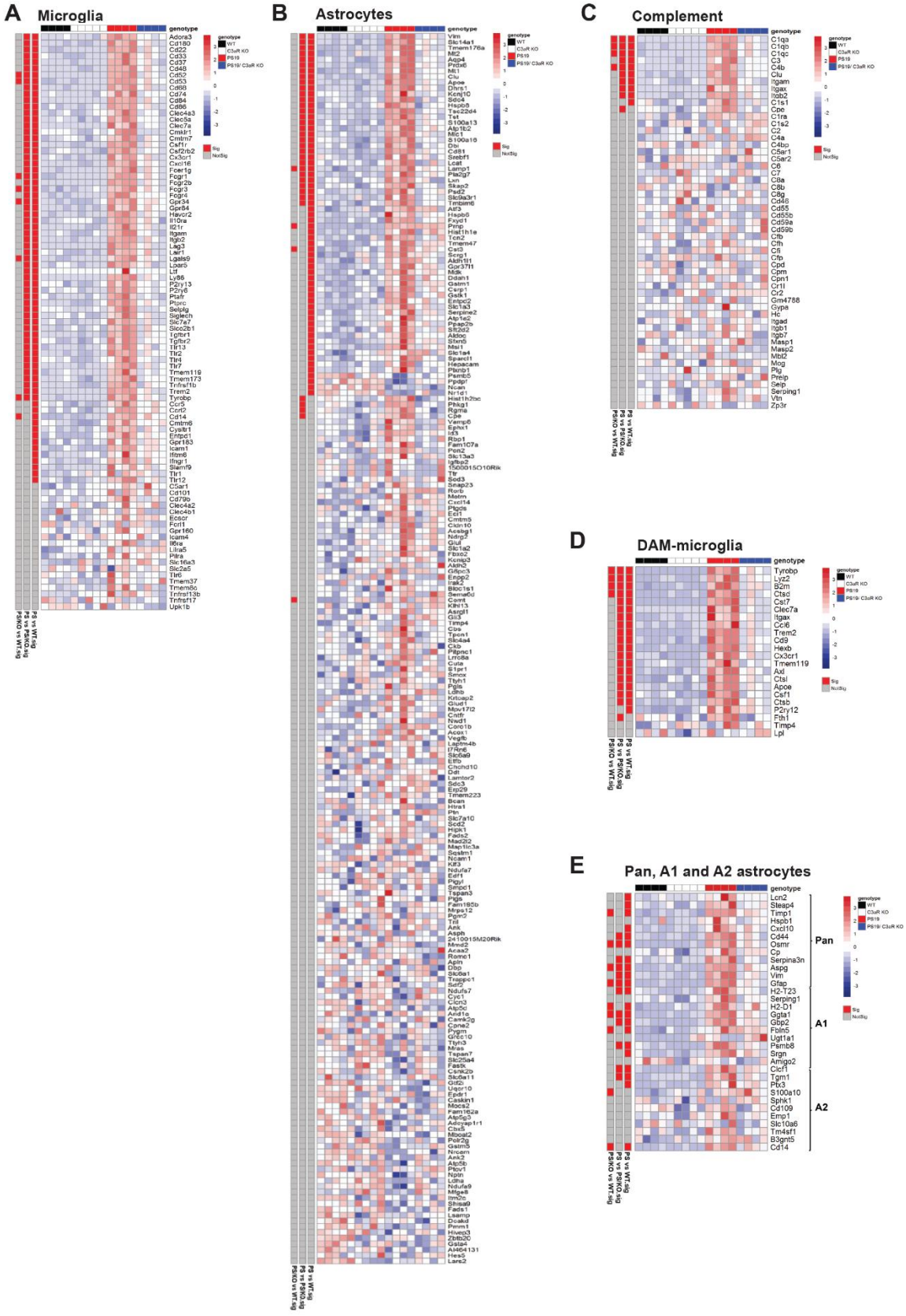

A
Microglia
B
Astrocytes
C
Complement
D
DAM-microglia
E
Pan, A1 and A2 astrocytes
genotype
WT
C3aR KO
PS19
PS19/ C3aR KO
Sig
NotSig
Pan
A1
A2

Figure S5. Related to Figure 6. C3aR regulates glial cell and complement pathway genes in PS19 mice. (A-C) Heatmaps of microglia sensome genes (A), reactive astrocyte genes (B), and complement pathway genes (C) from hippocampi RNAseq of 9 month-old WT, C3aR KO, PS19, and PS19/C3aR KO .

(D, E) Heatmaps of DAM markers (D) and A1-A2 astrocyte genes (E) obtained by hippocampal RNAseq. n=4/genotype.

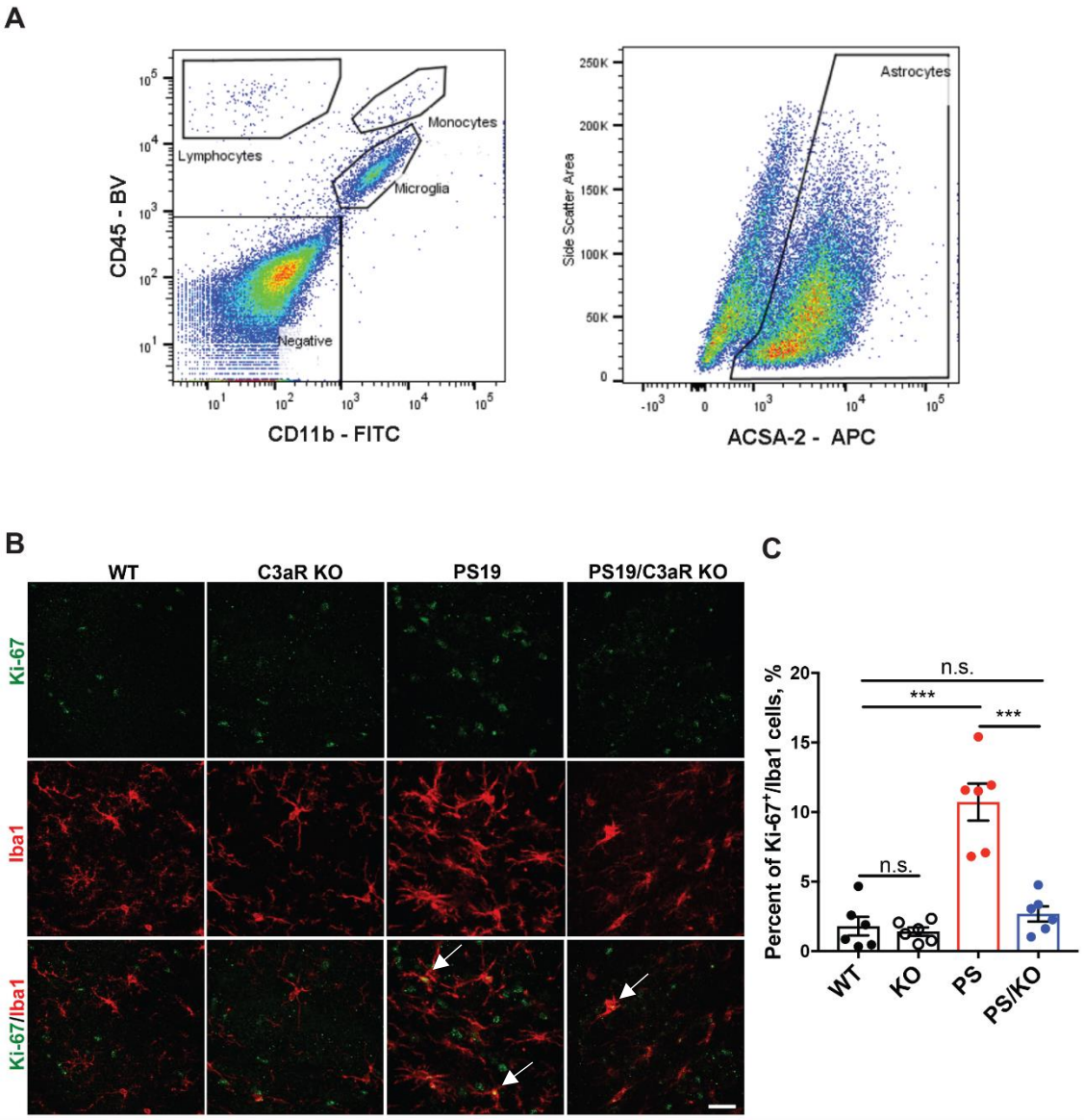

A
Lymphocytes
Monocytes
Microglia
Negative
CD45 - BV
CD11b - FITC
Astrocytes
Side Scatter Area
ACSA-2 - APC
B
WT
C3aR KO
PS19
PS19/C3aR KO
Ki-67
Iba1
Ki-67/Iba1
C
Percent of Ki-67+/Iba1 cells, %
n.s.
***
***
n.s.
WT
KO
PS
PS/KO

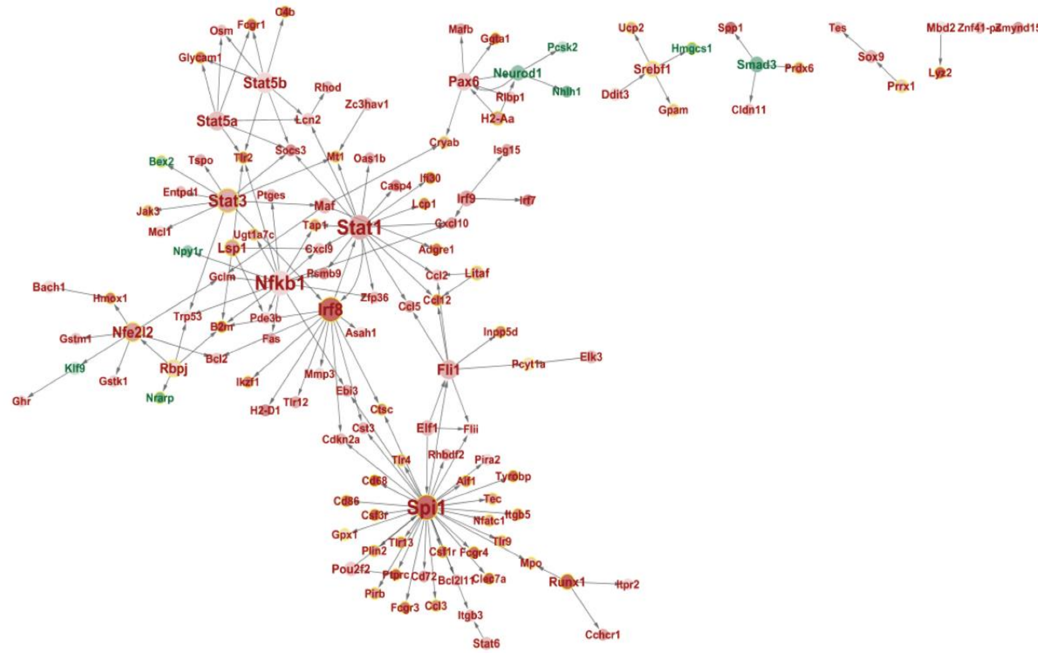
Figure S6. Related to Figure 6. *C3ar1* deficiency reduces microglial proliferation in PS19 mice.

(A) Representative flow plots of FACS-sorted CD45/CD11b^{+} microglia (left) and ACSA2^{+} astrocytes (right) from brain samples of 9 month-old PS19 mice.

(B) Representative co-immunostaining for Ki-67 and Iba1 in the hippocampus of 9 months WT, C3aR KO, PS19 and PS19/C3aR KO mice. Selective double positive cells are indicated by arrows. Scale bar: 50 µM.

(C) Quantification (B). n=6/genotype. One-way ANOVA followed by Sidak`s HSD test. n.s.: non-significant; ***p<0.001. Data is presented as mean ± s.e.m.

A


Stat1
Nfkb1
Irf8
Stat3
Stat5a
Stat5b
Spi1
Nfe2l2
Rbpj
Fli1
Runx1
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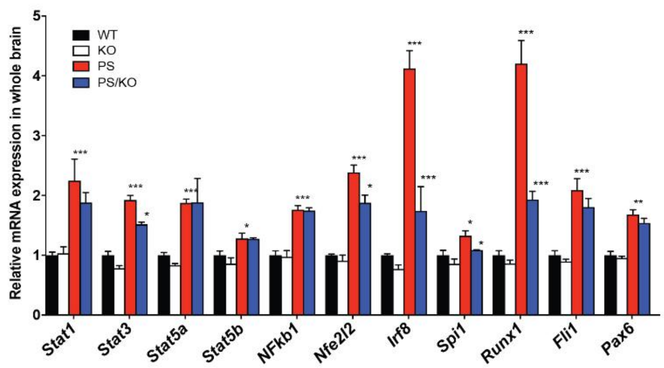
Neurod1
Srebf1
Smad3
Sox9
Lsp1
Maf
Irf9
Elf1

B


Relative mRNA expression in whole brain
WT
KO
PS
PS/KO
Stat1
Stat3
Stat5a
Stat5b
NFkb1
Nfe2l2
Irf8
Spi1
Runx1
Fli1
Pax6

C

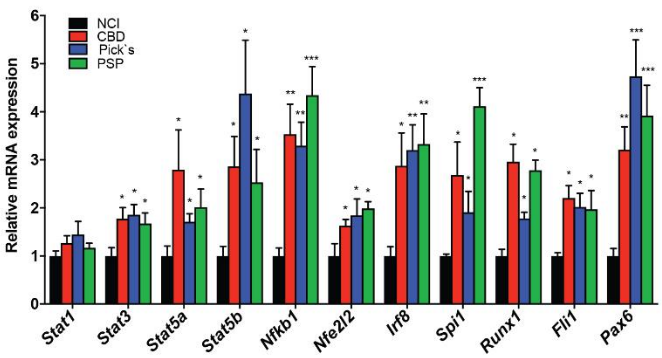

Relative mRNA expression
NCI
CBD
Pick`s
PSP
Stat1
Stat3
Stat5a
Stat5b
Nfkb1
Nfe2l2
Irf8
Spi1
Runx1
Fli1
Pax6

Figure S7. Related to Figure 6. Expanded TRANSFAC analysis and target validation.
(A) The transcription factor network of 1726 DEGs in PS19 vs WT comparison generated using TRANSFAC database with all the gene names displayed. Hub genes in the network denote the TFs that regulate the transcription of DEGs in PS19. Other network nodes represent the TF`s downstream targets. The size of the node corresponds to the number of connections. Color of the node: red - upregulated, green - downregulated in PS19. Genes rescued by C3aR KO in PS19 are highlighted by yellow circle around the node.
(B) qPCR analysis of mRNA levels of the TFs in a separate cohort of WT, C3aR KO, PS19 and PS19/C3aR KO mice. n=6 per group.
(C) qPCR analysis of the TFs in human tauopathy brain samples: NCI (n=6), CBD (n=5), Pick`s (n=5), and PSP (n=5). Two-way ANOVA followed by Tukey`s HSD test. *p<0.05; **p<0.01; ***p<0.001. Data is presented as mean ± s.e.m.

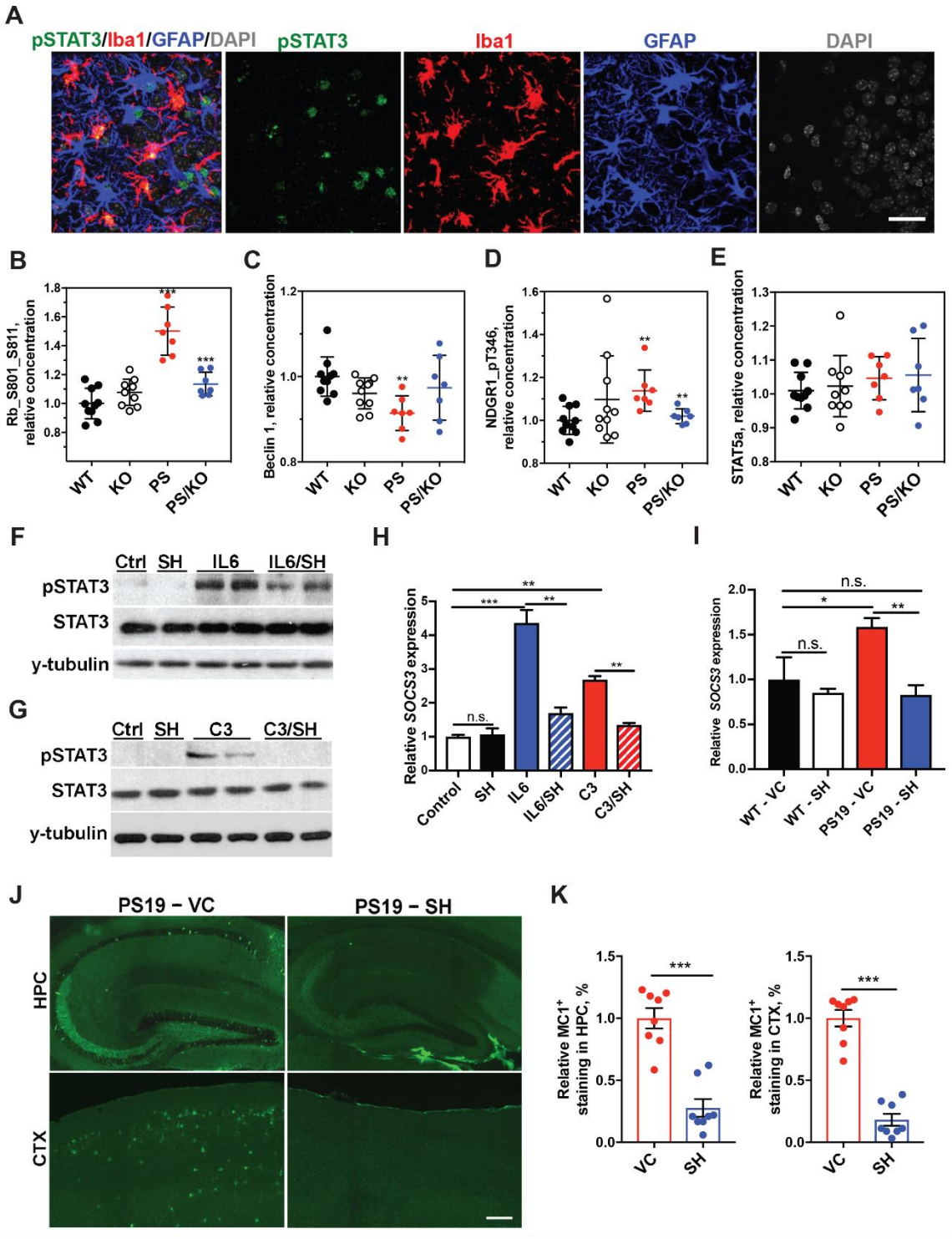

A
pSTAT3/Iba1/GFAP/DAPI
pSTAT3
Iba1
GFAP
DAPI
B
Rb_S801_S811, relative concentration
C
Beclin 1, relative concentration
D
NDGR1_pT346, relative concentration
E
STAT5a, relative concentration
WT
KO
PS
PS/KO
F
Ctrl
SH
IL6
IL6/SH
pSTAT3
STAT3
y-tubulin
G
Ctrl
SH
C3
C3/SH
pSTAT3
STAT3
y-tubulin
H
Relative SOCS3 expression
n.s.
Control
SH
IL6
IL6/SH
C3
C3/SH
I
Relative SOCS3 expression
n.s.
WT - VC
WT - SH
PS19 - VC
PS19 - SH
J
PS19 – VC
PS19 – SH
HPC
CTX
K
Relative MC1+ staining in HPC, %
Relative MC1+ staining in CTX, %
VC
SH

Figure S8. Related to Figure 7 and Figure 8. Expanded pSTAT3 and RPPA analysis.
(A) Co-immunostaining of pSTAT3 (Tyr705), Iba1, GFAP, and DAPI in the hippocampus of 9 month-old PS19 mice. Merged image reveal co-localization of pSTAT3 with both GFAP and Iba1.

(B-E) Other targets identified by RPPA showing significant changes in PS19 group compared to WT and opposite changed in PS19/C3aR KO compared to PS19. (B) pRb, (C) Beclin1, and (D) pNDGR1. In contrast to STAT3, STAT5a is indistinguishable across genotypes (E). The asterisks above PS19 are for PS19 vs WT comparison; those above PS19/C3aR KO are for the PS19 vs PS19/C3aR KO comparison. n=7-10/genotype. Student`s t-test. *p<0.01; **p<0.01.

(F,G) Western blot analysis showing that STAT3 inhibitor SH-4-54 (SH) blocks both IL6-induced (F) and C3-induced (G) STAT3 phosphorylation in BV2 cells.

(H) qPCR analysis showing SH-4-54 blocks IL6- and C3-induced *SOCS3* expression in BV2 cells. One-way ANOVA followed by Sidak`s HSD test. n.s., non-significant; **p <0.01; ***p<0.001.

(I) qPCR analysis showing SH-4-54 treatment downregulates *SOCS3* mRNA levels in PS19 but not in WT mice. VC - vehicle control, SH - SH-4-54. n=4/genotype. One-way ANOVA followed by Sidak`s HSD test. n.s., non-significant; *p<0.05; **p <0.01.

(J,K) Representative MC1 immunostaining (J) and quantification (K) in the hippocampus (HPC) and cortex (CTX) of 9 month-old vehicle (VC) or SH-4-54 (SH) treated mice. n=8/genotype/treatment. Student`s t-test. Scale bar: 0.5 mm. ***p<0.001.

All in vitro experiments (F-H) were repeated three times each in triplicates. All data is presented as mean ± s.e.m.